REMARKS

The cross-reference has been amended as suggested by the Examiner. Claims 14 and 31 have been amended, claims 15-16 and 18-19 cancelled, and new claims 34-37 added. Claims 14, 17, and 20-37 are presently pending. The amendments to the claims are fully supported by the original specification and claims. Specifically, the amendment to claim 31 and new claim 35 are supported at page 12, first paragraph; claim 14 and new claims 34 and 36 are supported at page 7, line 42 to page 8, line 8; and new claim 37 at page 8, line 12. No new matter has been added herein. Applicants therefore respectfully request that the amendments be entered at this time.

Applicants have noted that the Examiner has not received a certified copy of the priority document and in response have ordered a certified copy. When received, Applicants will immediately forward the certified copy to the Examiner. Furthermore, Applicants have amended the specification to clarify that the application is a continuation of the international application PCT/EP99/07168. Applicants note, however, that the Examiner has requested that that the earlier filed foreign application should be listed in the first paragraph of the instant application. Applicants respectfully point out that such an amendment would be improper according to the MPEP.

Claims 14, 16, 17, 31 and 32 were rejected under 35 U.S.C. Section 102(e) as being anticipated by U.S. Patent No. 6,143,563 to Peterson ("the '563 patent") for the reasons set forth on pages 3-4 of the office action.

The presently claimed invention of claims 14, 17, and 36-37 require that the primary regeneration tissue be dehydrated in a two-step process, with sequential incubation in stepped increase sucrose media. In particular, the primary regeneration tissue is first incubated in a medium containing 0.4 M sucrose followed by the incubation of the primary regeneration tissue in a medium containing 1 M sucrose. These claims further require the step of prefreezing the primary regenerating tissue to a temperature between -20°C and -40°C. The '563 patent neither teaches the specific disclosed concentrations of sucrose nor a stepped dehydration process of a sample to be cryo-frozen. The paragraphs cited by the Examiner merely give single concentrations of a specific osmoticum to be used, but do not teach stepped increases of sucrose. In addition, the '563 patent does not teach the prefreezing step as set forth as required by claims 14, 17, and 36-37.

The presently claimed invention as set forth in claims 31 and 32 requires the step of pretreating the primary regeneration tissue by culturing the primary regeneration tissue on multiple culture media with a increased concentrations of sucrose.

As mentioned above, the '563 patent fails to teach a pretreating step of culturing the primary regeneration tissue on multiple culture media with increased concentrations of sucrose *e.g.*, (0.25M/3d; 0.5M/5d; 0.75M/5d; 1.0M/2d).

In view of the '563 patent's failure to teach the required steps of the presently claimed invention, Applicants request that the rejection for anticipation be withdrawn.

Claims 14, 16-19, 24, 26, and 31-33 were rejected under 35 U.S.C. §103(a) as being unpatentable over the '563 patent to Peterson.

The Examiner argues in the office action that the "embroygenic callus" of the '563 patent is the same plant material as "primary regeneration tissue comprising embryogenic cells." The Examiner states that "although the second type of callus might demonstrate some early somatic embryo morphology, it is not a somatic embryo as taught by the cited patent." Applicants disagree.

Applicants point out at column 5, lines 13-15, of the '563 patent that the Type II callus consist of clearly formed somatic embryos often consisting of a somatic embryo bome on a suspensor-like structure. Primary regeneration tissue is tissue that gives rise to somatic embryos; the regeneration tissue comprises embryogenic cells. Once the tissue produces mature somatic embryos, it is no longer a primary regeneration tissue. One skilled in the art would understand the meaning of the term "primary regeneration tissue" accordingly. It is clear from the '563 patent that Peterson did not appreciate the advantages of culturing primary regeneration tissue as compared to other callus cultures. It was Applicants who discovered that culturing primary regeneration tissue comprising embryogenic cells provided increased regeneration rates compared to other tissue and callus cultures. The '563 patent did not disclose or appreciate these advantages.

The Examiner further argues that the '563 patent teaches a prefreezing step, citing (col. 2, lines 20-23 and col. 6, lines 26-34). Applicants respectfully traverse.

Column 2, lines 20-23, of the '563 patent is directed to the background art, wherein the '563 patent teach away from the presently claimed invention, stating in the next sentence that "[s]uch methods require an expensive programmable freezer to obtain adequate results." Furthermore, at column 6, lines 26-34 of the '563 patent cited by the Examiner is not directed

to a pre-freezing step, but is directed to alternative freezing temperatures for the step of cryofreezing if long term storage is not desired. There is no suggestion that a prefreezing step should be included in such a process and in fact, as mentioned above, the '563 patent, actually expressly teaches away from it. Applicants' presently claimed invention requires prefreezing of the primary regeneration tissue before cryofreezing. Applicants set forth in example 1, the unexpected advantages of prefreezing of the primary regeneration tissue prior to cryo-preservation.

Furthermore, the '563 patent also fails to disclose a pretreating step of the primary regeneration tissue on multiple culture media with an increased concentration of sucrose, *e.g.*, (0.25M/3d; 0.5M/5d; 0.75M/5d; 1.0M/2d).

The Examiner also argues that the '563 patent teaches the use of sucrose in induction medium and the use of increase concentration of sucrose in the multiplication medium. The paragraphs cited by the Examiner merely give single concentrations of a specific osmoticum to be used, but do not teach stepped increases of sucrose. Applicants provide and disclose the advantages of pretreatment in the culturing primary regeneration tissue in Examples 2 and 4. These examples show that dehydration/pretreating provides a significant increase in the regeneration rates of explants from the primary regeneration tissue.

It was Applicants that taught and first disclosed the advantages of culturing primary regeneration tissue and the need to pretreat and/or prefreeze the primary regeneration tissue prior to cryo-preservation to obtain increased regeneration rates. Applicants demonstrated that by using primary regeneration tissue in their specific process of cryo-preservation, the regeneration rate could be greatly increased and somaclonal variations could be decreased. The '563 patent does not suggest a preferential difference between what tissue should be cryopreserved and actually teaches that the steps of prefreezing and pretreatment are not necessary and should be avoided to save time and money.

In view of the above, Applicants respectfully request that the rejection for obviousness over the '563 patent be withdrawn.

Claims 14-33 were rejected under 35 U.S.C. §103(a) as being unpatentable over the '563 patent as applied to claims 14, 16-19, 24, 26, and 31-33 above, and further in view of Pence et al., Cryopreservation of immature embryos of Theobroma cacao, *Plant Cell Report*, Vol. 10, pp. 144-147 (1991); Ducos et al., U.S. Patent No. 5,943,821 ("Ducos"); and

Zimmerman et al., U.S. Patent No. 5,922,929 ("Zimmerman") for the reasons set forth on pages 6-9 of the office action. Applicants traverse.

None of the additional art cited by the Examiner eliminates the deficiencies of the '563 patent. Ducos, Zimmerman, and Pence are relied on solely for the teaching that callus cultures of any plant species can be subjected to cryopreservation, and specifically those of the plant species *Theobroma cacao*, *Coffee canephora*, or *Daucus carota*.

As stated above, the '563 patent fails to teach the steps of prefreezing and/or pretreating the primary regeneration tissue prior to cryofreezing and also fails to disclose the advantage of culturing primary regeneration tissue. None of the additional art cited by the Examiner remedies these deficiencies. Therefore, Applicants respectfully request that the obviousness rejection be withdrawn.

In view of the foregoing remarks and amendments it is believed that the entire application is now in condition for allowance. Should any issues remain the Applicants would like to request an in-person interview to resolve them. Please feel free to call Allan Fanucci at (212) 294-3311 or Rodney Fuller at (202) 371-5838 if you have any questions to expedite the allowance of all the claims in this application.

Respectfully submitted,

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